AD_____

HUMAN IMMUNE RESPONSES TO HTLV-III VIRUS INFECTIONS IN THE ACQUIRED IMMUNODEFICIENCY SYNDROME

ANNUAL REPORT

FRANCIS A. ENNIS

OCTOBER 28, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6279

Department of Medicine
Molecular Genetics and Microbiology
University of Massachusetts Medical Center
Worcester, MA 01605

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Department of Medicine Molecular Genetics and Microbiology; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): University of Mass. Medical Center Worcester, MA 01605	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research and Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-86-C-6279
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, M.D. 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 63105A; PROJECT NO. 3M2-63105DH29; TASK NO. AD; WORK UNIT ACCESSION NO. 019

11. TITLE (Include Security Classification): Human Immune Responses to HIV-1 Virus Infections in AIDS

12. PERSONAL AUTHOR(S): Ennis, Francis, A.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 10/86 TO 10/87	1987, October 28	10

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	03		AIDS; HIV-1; Retrovirus; Lentivirus; Immunology;
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of the research program is to define immune responses of humans to Human Immunodeficiency Virus (HIV) infection and the cause of the Acquired Immune Deficiency Syndrome (AIDS). Immunodeficiency caused by HIV infections may be due to immune mediated lysis (by antibody and/or HIV specific cytotoxic T lymphocytes [CTL]) of infected macrophages and T4 lymphocytes. The definitions of these immune responses to HIV virus are needed for rational approaches for therapy and prevention.

Since the project began in October, 1986, we have developed an integrated program of research projects to develop experimental systems required for defining the immune responses of humans to HIV.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

A. Summary

The purpose of the research program is to define immune responses of humans to Human Immuncdeficiency Virus (HIV) infection and the cause of the Acquired Immune Deficiency Syndrome (AIDS). Immunodeficiency caused by HIV infections may be due to immune mediated lysis (by antibody and/or HIV specific cytotoxic T lymphocytes [CTL]) of infected macrophages and T4 lymphocytes. The definitions of these immune responses to HIV virus are needed for rational approaches for therapy and prevention.

Since the project began in October, 1986, we have developed an integrated program of research projects to develop experimental systems required for defining the immune responses of humans to HIV.

B. Foreword

a) Copyright material - NA

b) Limited distribution material - NA

c) Commercial crganizations,etc - NA

d) Animal experimentation - NA

e) For the protection of human subjects the investigators have adhered to policies of applicable Law 45CFR46.

f) The investigators have abided by the NIH Guidelines for Research involving recombinant DNA Molecules (April 1982) and the Administrative Practices Supplement.

Table of Contents

C. Body of Report

1. Personnel

We have recruited into this new research program, Dr. Akira Takeda and Dr. Penny Jaffe as well as a professional technologist, Ms. P. Bulger.

a. Penny Jaffe, M.D. is a board certified specialist in internal medicine, and board eligible in infectious diseases. She worked on clonal T cell responses to influenza virus during a three year fellowship supported by the NIH. She has begun experiments which are designed to detect HIV virus specific T memory cell responses. These experiments are underway and preliminary results indicate that concentrated live HIV stimulates the peripheral blood mononuclear cells (PBMC) of HIV antibody positive donors, but not the PBMC of HIV antibody negative donors. This assay is the foundation upon which we will develop positive and negative controls for our future studies to define the virus specificity, including mapping the epitopes on HIV virus, which interact with T cells, in the human T cell responses to HIV.

b. Akira Takeda, M.D.

Dr. Takeda had previous research training on immunoregulatory mechanisms in human connective tissue diseases. He is establishing the HIV specific CTL assay. He has developed HLA-typed EBV transformed B cell lines from the PBMC of <u>HIV antibody positive donors</u>, aided by Ms. Bulger. He has also established HLA typed CD4+ enriched cell lines, as well as EBV transformed B cell lines from a panel of HLA-typed <u>HIV antibody negative</u> donors. These cell lines will be used as HIV infected, or Vaccinia-HIV antigenic hybrid virus infected, target cells for detecting HIV-specific CTL activity.

c. Ms. P. Bulger

Ms. Bulger is a Professional Technologist with considerable experience in producing murine monoclonal antibodies to protein antigens. She has begun studies to prepare human monoclonal antibodies using EBV transformed B cells from HIV antibody positive donors. These studies will be difficult but are important in order for us to identify antibody combining sites on HIV, and the role of antibody on antigenic variation of the virus. To date nine EBV transformed cell lines have been established from HIV antibody positive donors, and the first four donors EBV-transformed PBL are being analyzed at present for the production of HIV specific antibodies.

Our efforts in the first year of the proposal including the following:

2. Obtaining and cryopreserving peripheral blood mononuclear cells

a. <u>HIV antibody negative donors</u>

We have obtained the PBMC from a number of HLA-typed HIV antibody negative donors. We now have approximately 20 HLA-typed donors' PBL cryopreserved in liquid nitrogen from HIV antibody negative normal blood bank donors. These buffy coats were obtained as part of platelet donation at the blood bank here at the University of Massachusetts.

b. PBMC from HIV-antibody positive donors

In addition, we have obtained the PBMC of 14 HIV antibody positive donors obtained from collaborators at George Washington University School of Medicine (Dr. Carmen Tuazon, Professor of Medicine) and at Walter Reed Army Medical Center (Dr. Craig Wright and Dr. Joanne Rhoades).

3. Virus and Virus infected cell lines.

H9 cells infected with the HTLV-IIIB virus and uninfected cells were obtained from Dr. R.C. Gallo. Live concentrated HTLV-III_B virus grown in Molt-3 cells has been obtained from Dr. Claudine Bruck.

4. Experiments have been initiated to develop a panel of EBV transformed lymphoblastoid cell lines from each of the HIV antibody positive donors. (see above)

5. Experiments have been initiated to demonstrate HIV specific T cell memory in the PBLs of the preantibody positive or antibody negative donors. (see Table 1 below for preliminary data)

6. Experiments are underway to identify enhancing antibodies in the sera of HIV infected individuals and to determine their effect on HIV replication in human monocytic cells, similar to our experiments using dengue virus infected human monocytes. (See table 2 below for preliminary data.)

Table 1. HIV induced proliferation of PBMC is determined by ^3H thymidine incorporation

Donor Ab pos #2	Mean[a] CPM	Stim Index	Donor Ab neg #HJ	Mean CPM	Stim Index
Media	254	1	Media	187	1
HIV:5X	6,473	25	HIV 5X	192	1
1X	3,911	15	1X	210	1
.1X	1,136	4	.1X	192	1
.01X	537	2			
PHA	33,731		PHA	48,206	
# 12					
Media	263	1			
HIV 5X	1,453	5			
X	682	2.6			
.1X	552	2.1			
PHA	82,106				

[a]Mean CPM of 4 microtiter wells each containing 2 x 10^5 cells/well in 100 ul. On day 0 an equal volume of medium alone or infectious HIV virus grown in MOLT-3 cells and concentrated on a metrizamide gradient (provided by Dr. C Bruck) was added. A ^3H thymidine pulse was performed on day 5, and cells were harvested by a MASH harvester after overnight incubation.

The results shown in Table 1 indicate we have established methods for stimulating HIV specific memory T cells. In other experiments, we have shown that the PBL HIV antibody positive donor #12 have reproducible, moderately high levels of HIV specific proliferation, and these PBL have been our positive control. We have also shown that the PBL of donors who are HIV antibody negative (0/4 tested) do not respond to this HIV antigen. We will attempt to restimulate the PBL of high responding donors to develop HIV specific T cell lines/clones in order to eventually define the sites on HIV which induce T cell responses, and to characterize the T cells involved.

The EBV transformed autologous B cell line may be useful in developing such T cell clones, since the restimulation of PBL with HIV antigens will require autologous antigen presenting cells. We realize the autclogous B cell lines are likely to induce T cell responses to EBV antigens from the the PBL of most of the donors. We will determine whether any of the HIV antibody positive donors lack EBV antibodies. We can also try limiting dilution cloning early after stimulation in an effort to select out HIV responding T cells from EBV responding clones. We will also study these HIV specific proliferating T cells for CTL activity on autologous EBV transformed B cells infected with HIV, or vaccinia-HIV hybrid viruses, which Walker, et al recently reported were useful for directly detecting HIV specific CTL, without in vitro stimulation. We have obtained the vaccinia-HIV hybrids from B. Moss and C. Bruck, and relevant laboratory personnel were recently revaccinated with smallpox vaccine, as recommended by the CDC which supplied the vaccine. Experiments will be performed in the coming year to determine which of our donors have HIV specific CTL directly detectable on autologous HIV infected or vaccinia-HIV infected target cells, as described above, and the effect of restimulation on CTL activity. These studies are important in order to understand the role and specificity of HIV specific T cells in the pathogenesis, and eventually in prevention of AIDS

We have begun to investigate the role of monocytic cells in HIV infection based on our work, and the work of others, in flavivirus infections. We began experiments to examine certain characteristics of HIV infection of human monocytes, using U937 cells as a model system. Monocytic cells can be infected with HIV, and human monocytes might be suitable as autologous, or HLA matched, target cells for HIV specific CTL investigations. During the course of these studies, we have obtained data concerning the effects of neutralizing antibodies and, at higher dilutions of the same sera, enhancing antibodies on the ability of HIV to infect these Fc receptor bearing cells.

Representative results from an experiment are shown below in Table 2. The results in the Table illustrate that treatment of HIV with human serum without antibodies to HIV did not change the number of cells infected with HIV. Human sera, however, which are known to contain antibodies by HIV (by Elisa and Western blot), decreased the number of HIV infected cells at lower serum dilutions (e.g. 1:1 -1:100), but appeared to increase the number of infected cells at higher dilutions. These studies are being actively pursued, because the enhancement of infection of Fc receptor bearing cells by HIV ag-ab complexes may be important in the pathogenesis of AIDS.

Table 2. Antibody mediated enhancement of HIV infection of Human Monocytic Cells (U937)

HIV[a]	Ab[b]	Dil'n	Percent of Cells infected with HIV					
			Day 10			Day 11		
			#+	Total	% positive	#+	Total	% positive
-	-		0	109	0	0	110	0
+	-		66	206	32.0	230	443	529
+	Non-immune	1:10	64	224	28.6	109	245	44.5
+	"	1:100	68	229	29.7	122	219	55.7
+	"	1:1000	66	243	27.1	124	255	48.6
+	"	1:10,000	78	262	29.8	113	217	52.1
+	HIV Ab pos	1:10	32	455	7.0	17	235	7.2
+	#	1:100	4	263	1.5	7	212	3.3
+		1:1000	47	223	21.3	142	306	46.4
+		1:10000	98	286	34.3	167	252	66.3
+	HIV Ab pos	1:10	3	287	1.0	12	240	5.0
+	#	1:100	87	234	37.2	111	162	68.5
+		1:1,000	63	229	27.5	94	187	50.3
+		1:10,000	101	223	43.5	105	132	79.5

[a]HIV-HTLV III_B virus obtained from H9 infected cell supernatants was added to diluted sera[b] for 2 hours at 4°C before being added to U937 cells. After 2 hours of incubation with virus-antisera mixtures, the U937 cells were washed x 2 and resuspended in 10% FCS and RPMI 1640 and were incubated at 37°. Aliquots of cells were removed on days 9 & 10 and tested by indirect flourescent antibody using a human serum (supplied by Dr. R. Redfield) known to be positive by ELISA and Western blot for HIV.